Difference in Early Effects of Statin Therapy on Coronary and Forearm Flow Reserve in Postmenopausal Hypercholesterolemic Women

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Background The relationships between flow reserves in coronary and peripheral circulation and their modification by statin therapy have not been assessed in the same patients, especially females.

Methods and Results To assess the effect of pravastatin on both circulation, in 20 postmenopausal female patients with hypercholesterolemia but a low probability of coronary artery disease, the forearm blood flow reserve (FBFR) using the plethysmographic method and coronary flow velocity reserve (CFVR) by Doppler echocardiography were measured before, 4 and 8 weeks after starting the pravastatin and/or diet therapy. At baseline, CFVR and FBFR had a positive linear correlation ($r=0.63$, $p<0.01$) while each of them had a negative linear correlation ($r=-0.53$ to -0.63 , $p<0.05$ each) with total or LDL-cholesterol levels. Four weeks after starting the pravastatin therapy when the decrease in total cholesterol reached a plateau, FBFR increased ($p<0.05$) by $38\pm14\%$, whereas CFVR did not. Such an increase in FBFR by pravastatin was related to the degree of total or LDL-cholesterol lowering.

Conclusions In postmenopausal hypercholesterolemic women with a low probability of coronary artery disease, the cholesterol-lowering with pravastatin improved FBFR as early as 4 to 8 weeks after starting the therapy, whereas its effect on CFVR was unclear at that time. (*Circ J* 2007; 71: 954–961)

Key Words: Coronary circulation; Flow reserve; Hypercholesterolemia; Peripheral circulation; Women

There is a correlation of endothelium-dependent vasodilating function between coronary and peripheral circulation.¹ To assume coronary endothelium-dependent vasodilatation, an assessment of peripheral circulatory function in the forearm or brachial arteries needs to be done. In contrast, microcirculatory responses to vasodilating stimuli might somewhat differ between coronary and peripheral circulation. Thus, peripheral flow reserve assessed by reactive hyperemia did not correlate with the coronary flow reserve assessed by adenosine administration in the mixed population of the normal control, known coronary heart disease (CHD), and cardiac syndrome X.² However, the possible influence of epicardial coronary stenosis on coronary flow reserve was not determined in those CHD patients. In contrast, a cholesterol-lowering therapy with statin ameliorated decreases in peripheral³ or coronary flow reserve⁴ in hypercholesterolemic patients. In the latter study,⁴ an assessment of coronary flow reserve was conducted 6–12 months after starting statin therapy. Thus, the correlation of coronary and peripheral flow reserves and their modification by statin therapy has not been assessed in detail; that is, simultaneous assessment in the same subjects with a low probability of CHD remains to be investigated, especially in the early phase after start-

ing statin therapy. Further, it still remains to be determined whether flow reserve on peripheral microcirculation is helpful in predicting that on coronary microcirculation to assess the therapeutic effects of statin.

The cholesterol-lowering interventions reduce the risk of CHD^{5,6} and mortality.^{7–10} In primary prevention studies^{7–9} or assessment of changes in vascular function by therapies,^{11,12} male subjects have been the predominant study population. Because the risk of CHD increases after menopause in females, there is a need to assess whether the cholesterol-lowering therapy with statin is beneficial in terms of the primary prevention of CHD in hypercholesterolemic postmenopausal women with a low probability of CHD.¹³ Among a few reports on this issue, the significant effects of pravastatin on primary prevention of CHD in female subjects with mild to moderate hypercholesterolemia treated with pravastatin were recently presented at the American College of Cardiology Meeting in 2006 as subanalytical results of the MEGA study.¹⁴

The purpose of the present study is to investigate: (1) whether coronary and peripheral flow reserves and their correlation are modified in hypercholesterolemic postmenopausal women with a low probability of CHD; and (2) whether cholesterol-lowering therapy with pravastatin affects coronary flow reserve and forearm blood flow reserve (FBFR) soon on after starting the therapy (in those subjects).

Methods

Patient Population

Twenty postmenopausal female patients with hypercho-

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Table 1 Baseline Characteristics of Subjects

	Normal control (non-HC) group (n=10)	Hypercholesterolemic (HC) group		
		Sum (n=20)	With statin (n=10)	Without statin (n=10)
Age (years)	57±2	61±1	61±2	60±1
BMI	22±1	23±1	23±1	22±1
SBP (mmHg)	131±4	128±3	126±5	130±4
DBP (mmHg)	82±3	82±3	83±3	80±2
HR (beats/min)	64±5	63±3	63±2	62±2
Hypertension [n (%)]	0 (0)	6 (30)	3 (30)	3 (33)

Data are mean ± SE.

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.

Table 2 Serum Lipids and Glucose Levels in Normal Controls (non-HC) (n=10) and Hypercholesterolemic (HC) Patients (n=20)

Serum parameter groups	Baseline	4 weeks after starting therapies	8 weeks after starting therapies
Total cholesterol (mg/dl)			
Non-HC	187±6		
HC	260±7*	235±6***	238±6***
With statin	268±7*	232±10***	234±8***
Without statin	253±12*	237±9*	242±9*
Low-density lipoprotein-cholesterol (mg/dl)			
Non-HC	107±7		
HC	169±7*	144±8***	150±7*†
With statin	172±6*	140±9***	143±8***
Without statin	162±12*	150±14*	156±12*
High-density lipoprotein-cholesterol (mg/dl)			
Non-HC	66±4		
HC	57±3	60±4	58±3
With statin	59±4	61±7	57±6
Without statin	55±3	59±4	58±4
Triglyceride (mg/dl)			
Non-HC	92±11		
HC	164±30*	164±39*	156±41*
With statin	161±17*	162±25*	140±29*
Without statin	168±60*	165±80*	168±75*
Blood sugar (mg/dl)			
Non-HC	90±2		
HC	94±1	96±3	93±2
With statin	93±2	95±3	94±3
Without statin	95±1	96±3	91±1

Data are mean ± SE.

* $p < 0.01$ vs baseline values of non-HC (normal control) group; ** $p < 0.01$; † $p < 0.05$ vs baseline values of corresponding group.

lesterolemia (HC group; with serum cholesterol level >220 mg/dl) and 10 age-matched non-hypercholesterolemic postmenopausal female volunteers (non-HC group) were enrolled in this study. The baseline characteristics of the subjects are shown in Tables 1 and 2. Smokers and those who were diabetic were not included in the study, and 30% of all subjects had untreated mild hypertension (Table 1). No subjects were taking any medication for any disease at the time of entry. In the HC group, a standard diet therapy was introduced by a medical doctor to all patients according to the recommendations of the National Cholesterol Education Program.¹⁵ In addition, patients were asked whether they wished to start their pharmaceutical therapy with statin. Ten out of 20 patients agreed to use statin (HC group with statin) while the other 10 did not (HC group without statin). Patients with documented CHD, heart failure, atherosclerotic vascular diseases, atrial fibrillation, other organic diseases, and chest pain suggestive of angina, were excluded from the study. Patients with any chest pain or discomfort (atypical or typical for CHD) at rest or on effort were also excluded. Although we had not performed further screen-

ing tests for CHD at the time of entry, we retrospectively confirmed that all patients were negative for ischemia on symptom-limited treadmill stress ECGs. Those who had been already taking cholesterol-lowering drugs were also excluded from the present study.

This prospective clinical study was approved by the in-hospital ethics committee and was also carried out according to the Declaration of Helsinki. Written informed consent was obtained from all subjects before they entered the study.

Study Protocol

All subjects who enrolled were right-handed. As the baseline study in all subjects, after overnight fasting, blood access was obtained from the left forearm veins and blood samples for biochemical analysis were taken. Measurements of the right FBFR were then performed by using the plethysmographic method, which is described later. Finally, the coronary flow velocity reserve (CFVR) was measured by the transthoracic pulsed Doppler echocardiography combined with an intravenous infusion of adenosine triphosphate, which is also described later.

Fig 1. Actual tracings of records or images on plethysmography and Doppler echocardiography at baseline, and during reactive hyperemia or ATP infusion in representative cases of the hypercholesterolemia (HC)- and non-HC group. TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; FBFR, forearm blood flow reserve; CFVR, coronary flow velocity reserve.

After the baseline study, the statin group and the diet group started a similar standard diet therapy for hypercholesterolemia. In addition, in 10 patients of the HC group, oral intake of pravastatin 10 mg/day once daily at night was started within a week. Four and 8 weeks after starting the therapies, blood sampling in the fasted state and same-day measurements of FBFR and CFVR were repeatedly performed.

Assessment of FBFR

In the postabsorptive state in a warm (23–27 Centigrade) and quiet room, the forearm blood flow was measured in the right arm by a mercury-in-silastic Whitney strain gauge plethysmography (EC 5R plethysmography™, D. E. Hokanson, Inc, USA) using the venous occlusion technique.¹⁶ The strain gauge was placed approximately 5 cm below the antecubital crease. The pressure in the venous occlusion cuff of the upper arm was 50 mmHg. Following the placement of the instruments, the baseline forearm blood flow was measured after a 15-min rest period during which patients were familiarized with the experimental techniques. The measurement was made every 15 s throughout the last 3 min at baseline, and the mean value was determined as a baseline value and expressed as milliliters per 100 ml of forearm tissue per min. Measurements in all subjects were conducted by the same doctor who was trained

in how to perform the plethysmographic method. The intra-observer variability (the standard deviation of the differences/average value) was $8 \pm 1\%$. To determine the forearm vasodilating capacity, the forearm blood flow was then measured after 5 min of arterial ischemia induced by inflation of the cuff on the upper arm under suprasystolic blood pressure (reactive hyperemia). After release of the arterial occlusion, the forearm blood flow was measured at 5 s (peak reactive hyperemia) and every 15 s thereafter for 2 min. The increased ratio in the forearm blood flow at the peak reactive hyperemia from the baseline value was considered as the FBFR.

Assessment of CFVR

Transthoracic Doppler echocardiography was performed with a digital ultrasound system (Aplio80™, Toshiba, Japan) with a frequency of 5.0 MHz. In color Doppler flow mapping, the velocity range was adjusted to provide optimal images.¹⁷ Acoustic windows were around the mid-clavicular line in the fourth and fifth intercostal spaces in the left lateral decubitus position. The ultrasound beam was transmitted toward the heart by color Doppler flow mapping to visualize coronary blood flow at the distal portion of the left anterior descending coronary artery (LAD). By positioning a sample volume on the color signals at the distal LAD portion, Doppler spectral tracings of flow velocity were

Fig 2. Relationships between serum lipid levels and forearm blood flow reserve (FBFR) or coronary flow velocity reserve (CFVR) in normal control and hypercholesterolemic subjects at baseline. Serum total cholesterol (TC) and low-density lipoprotein-cholesterol (LDL-C) had negative linear correlations with FBFR and CFVR.

recorded. At first, baseline spectral Doppler signals at the distal LAD portion were measured over 5 cardiac cycles at the end-expiratory phase by transthoracic Doppler echocardiography. The measurements in all subjects were performed by the same doctor, who is a board-certified fellow of the Japanese Society of Ultrasonics in Medicine. The intra-observer variability was $6 \pm 2\%$. After that, spectral Doppler signals were obtained during peak hyperemic conditions by an intravenous adenosine triphosphate ($140 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) infusion. CFVR was assessed as the ratio of peak hyperemic to basal averaged diastolic mean velocities.

Statistical Analysis

Data are expressed as mean $\pm$ SE. For multiple comparisons, 2-factor factorial ANOVA was performed. If F test results were < 0.05 , Bonferroni's post-hoc comparisons were performed. A p value < 0.05 was considered to be significant.

Results

Serum Lipid Levels

Compared to the non-HC group, serum total and low-density lipoprotein (LDL)-cholesterol levels in the HC group at baseline (measured before starting therapies) were higher ($p < 0.01$ each), whereas the high-density lipoprotein (HDL)-cholesterol and triglyceride levels tended to be lower (NS each), but blood glucose levels were similar

Fig 3. Relationship between coronary flow velocity reserve (CFVR) and forearm blood flow reserve (FBFR) in normal control and hypercholesterolemic subjects at baseline. There was a positive linear correlation between CFVR and FBFR. HC, hypercholesterolemia.

Fig 4. Percent (%) changes from baseline in serum total and low-density lipoprotein (LDL)-cholesterol (A and B) levels, forearm blood flow reserve (C) and coronary flow velocity reserve (D) over time after starting therapies. HC, hypercholesterolemia.

(Table 2). In the HC group without statin, total and LDL-cholesterol levels tended to be low (NS). Four and 8 weeks after starting the statin and diet therapies, total and LDL-cholesterol levels decreased ($p<0.01$) in the HC group compared with baseline values (measured before starting the therapies), whereas the HDL-cholesterol levels did not change. In addition, in this group, the serum triglyceride levels tended to be low (NS) at 8 weeks compared with baseline values. Four and 8 weeks after starting the diet therapy in the HC group without statin compared with baseline values, the total and LDL-cholesterol levels tended to be low (NS) while serum triglyceride levels were similar to the baseline values.

Relationships Between Serum Lipid Levels and Flow Reserves at Baseline

Actual tracings of records or images on plethysmography and Doppler echocardiography at baseline, and during reactive hyperemia or ATP infusion in representative cases of the HC- and non-HC group, are shown in Fig 1. The relationships between total or LDL-cholesterol levels and FBFR or CFVR of the non-HC, which is the HC groups with or without statin in the baseline study, are shown in Fig 2. Both total and LDL-cholesterol levels had negative linear correlations ($r=-0.53$ to -0.62 , $p<0.01$ each) with FBFR and CFVR, respectively.

Relationship Between Coronary and Forearm Flow Reserve at Baseline

The relationship between CFVR and FBFR of the non-HC, which is the HC group with or without statin in the baseline study, is shown in Fig 3. These values including those of the all 3 groups had a positive linear correlation ($r=0.60$, $p<0.01$).

Serial Changes in Serum Lipid Levels and Flow Reserves After Starting Therapies

In Fig 4, the changes are shown in total (A) and LDL-cholesterol (B) levels, FBFR (C), and CFVR (D) in the HC groups with or without statin from the baseline until 4 and 8 weeks after starting the therapies. In the HC group without statin, those 4 values did not change during these time-courses. In contrast, there were decreases in total cholesterol (by $12\pm2\%$ at 8 weeks) and LDL-cholesterol levels (by $17\pm3\%$ at 8 weeks) ($p<0.01$) in the HC group with statin at 4 and 8 weeks compared with the baseline values. In the HC group with statin, FBFR increased (by $52\pm17\%$ at 8 weeks, $p<0.01$) compared with the baseline values 4 and 8 weeks after the therapies, whereas CFVR did not.

Serial Changes in Relationships Between Serum Lipid Levels by Therapies and Flow Reserves

The mean $\pm$ SE values of total or LDL-cholesterol levels and of FBFR or CFVR before and 4 and 8 weeks after starting the therapies are plotted in Fig 5. Among the triple

Fig 5. Plots of relationships between serum lipid levels and forearm blood flow reserve (FBFR) (A and B) or coronary flow velocity reserve (CFVR) (C and D) in the non-hypercholesterolemic group (non-HC group) at baseline, and the hypercholesterolemic (HC) groups with or without statin (pravastatin administration combined with diet therapy) subgroups at baseline, and 4 and 8 weeks after starting therapies. Total cholesterol (TC) or low-density lipoprotein-cholesterol (LDL-C) levels decreased in the HC group with statin therapy. FBFR increased with negative linear correlations whereas there were no correlations between TC or LDL-C and CFVR. Solid and dotted lines in each Figure show the linear fitting line and 95% confidential intervals, respectively.

time-points measured, in parallel with decreases in total or LDL-cholesterol levels by the pravastatin administration, FBFR increased near linearly. Namely, there were negative linear correlations between total or LDL-cholesterol levels and FBFR among the 7 subgroups, suggesting that the increase in FBFR by statin therapy might depend on the degree of cholesterol lowering achieved. In contrast, despite significant decreases in total and LDL-cholesterol levels by the statin therapy, CFVR did not change among the 7 subgroups.

Discussion

Effects of Statin Therapy on Microcirculation

There have been no reports that have assessed coronary flow reserve and forearm flow reserve comparatively in the same subjects, and which also assessed the effects of statin therapy on both flow reserves. In the present study, there were negative linear correlations between total or LDL-cholesterol levels in the sera and FBFR or CFVR (Fig 1) and a positive linear correlation between FBFR and CFVR (Fig 3). These results suggest that hypercholesterolemia

impairs both coronary and forearm microcirculation. In contrast, the effects of the statin therapy on FBFR and CFVR were different. FBFR increased after statin therapy whereas CFVR did not change significantly. The results suggest that coronary or forearm microcirculation responds differently to statin therapy. In addition, the increase in FBFR by statin therapy seemed to depend on its total cholesterol- or LDL-cholesterol-lowering effect (Fig 5). This apparently might not be concordant with the results of a previous report in which pravastatin did not improve FBFR but fluvastatin did partly via its anti-oxidant effect, which is one of the statin's pleiotropic effects.⁴ The reasons for such different effects of pravastatin on FBFR between the 2 studies remain to be determined. Since both studies were performed in using the same race, that is, Japanese, and the severity of hypercholesterolemia was similar, the gender difference in subjects enrolled might be a factor. In contrast, in regard to improvement of forearm microcirculatory function, FBFR did not improve as much as expected from the degree of the total cholesterol- or LDL-cholesterol-lowering effect. Therefore, we were not able to confirm the effect beyond lipid lowering (ie, the so-called pleiotropic

effect) (Fig 5). To delineate the roles of the cholesterol lowering and statin's pleiotropic effect on FBFR responses, a controlled study using a cholesterol-lowering therapy other than statin will be required in future.

Effect of Statin on CFVR

We did not see an increase in CFVR from pravastatin therapy for 8 weeks. There are reports that the increase in coronary flow reserve by the statin required 6 months^{15,16} Simvastatin reportedly increased coronary flow reserve 10 months after starting the therapies whereas pravastatin did not.¹⁸ Although our study was aimed to investigate the short-term effects of pravastatin on both coronary and forearm microcirculation, taking into consideration the possibility of the late effect suggested by previous reports,^{19,20} a long-term follow-up study with the use of different cholesterol-lowering therapies including comparisons between pravastatin and other statins is warranted in the future.

Implication of Statin Therapy in Females

The results of the present study show a seeming contradiction with the subanalytical report of the MEGA study, which showed the primary prevention of CHD by pravastatin, as indicated in the Introduction. Our data showed a hypo-responsiveness of coronary microcirculation 8 weeks after starting pravastatin, in contrast to significant improvement of forearm microcirculation. Our limited results over a rather short-term period might not allow a comparison with those obtained from the primary prevention of CHD 5 years after starting the therapy. However, we can at least note that early improvement of forearm microcirculation by statin therapy does not necessarily imply early improvement of coronary microcirculation.

Effect of Diet Therapy

In the diet therapy group (HC group without statin), the baseline serum values of total- and LDL-cholesterol tended to be low (N.S.) compared with those of the HC group with statin. As our study was not designed for double-blinded assessment, it is possible that subjects with relatively lower total- and LDL-cholesterol levels chose not to use a statin.

The effects of the diet therapy on lowering total- and LDL-cholesterol levels were not significant in the present study (Fig 4). In a review of previous large-scale clinical trials, diet therapy tended to improve serum lipid levels but not significantly.² The results of the present study appear to be compatible with that report, although the small sample and short-term observation of effects are limitations. However, our results do not deny the possibility that more aggressive dietary intervention (eg, the educational hospitalization for diet therapy) might achieve significant cholesterol-lowering effects.

Possible Mechanisms of Decreased Flow Reserve in Hypercholesterolemia and Its Response to Pravastatin Therapy

Although microvascular vasodilatory capacity is composed of endothelium-independent and -dependent mechanisms, it is generally considered that the former mechanism is dominant.²¹ In addition, there seems to be a close correlation of endothelial function between coronary and peripheral circulation.²² Furthermore, FBFR had a negative linear correlation with the total- and LDL-cholesterol level (Fig 5). Therefore, we speculate that the different responses to the cholesterol lowering in maximal vasodilatory function in

microcirculation of coronary and forearm vascular beds might be largely attributed to the difference in endothelium-independent responses in both vascular beds, although its molecular mechanisms remain largely unknown. Recently, it has been reported that adenosine opens voltage-dependent potassium (Kv) channels²³ of smooth muscle cell in arterioles, resulting in activation of cAMP-dependent protein kinase A and microvessel relaxation, and hypercholesterolemia abolishes the tetraethylammonium (non-selective potassium channel blocker)-sensitive Kv channel contribution to adenosine-mediated dilatation of coronary arterioles.²⁴ If this novel action on hypercholesterolemia is different in forearm microcirculation, the different responses in microcirculation to the cholesterol-lowering therapy between coronary and forearm vascular beds might be partly explained.

Implications of the Study

Our results imply that improvement of forearm microcirculation can be expected early after starting pravastatin therapy in mild to moderate hypercholesterolemic female subjects with a low probability of CHD, whereas it may not help predict simultaneous improvement of coronary microcirculation in such an early stage. Also, in concert with the studies cited in this paper, cholesterol-lowering therapy may need to be continued for a certain long time when we aim to increase coronary flow reserve. Our results may also suggest more consideration of therapeutic strategies for ameliorating the hypercholesterolemia-induced reduction of coronary flow reserve.

Study Limitations

There are numerous study limitations. First, the number of subjects enrolled was quite low and was not randomized as mentioned above. Second, we did not compare the data obtained from female subjects with those from males. Therefore, it still remains to be determined whether different responses of CFVR and FBFR to the statin therapy are specific to females. Third, we assessed the effects of statin only using pravastatin. Another statin might have different effects on both vascular beds. Fourth, although serum triglyceride levels tended to be lowered by statin therapy, one cannot rule out such effects of triglyceride lowering on coronary and peripheral microcirculations. Fifth, our follow-up period after starting statin therapy was short. To assess the prognostic value of flow reserve modulation, long observation periods are required. And finally, the study was not randomized.

Thus, further study is required to assess the effects of pravastatin on hypercholesterolemic postmenopausal patients in regard to early responses of coronary and peripheral microcirculation.

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